SCIENTIFIC LETTER

Relation between nitric oxide metabolites and haemoglobin concentrations in patients with ischaemic heart disease

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Reduced haemoglobin concentrations ([Hb]) are associated with adverse cardiovascular outcomes in patients with acute coronary syndromes.[1] The reasons behind this association may include, in addition to impaired oxygen delivery,[1] chronic inflammation,[2] reduced erythropoietin activity,[1 3] or a general depression of haematopoietic function.[4] Indeed, an impoverished circulating reservoir of stem and progenitor cells (PCs) is emerging as a new indicator of cardiovascular risk.[4]

Nitric oxide (NO), well known for its vasodilatory and antiplatelet actions, is typically reduced in patients with ischaemic heart disease (IHD)[5] and may contribute to regulate [Hb]. The type II, cytokine-induced isoform of NO synthase causes haematopoietic suppression in vitro.[2] On the other hand, the endothelial (constitutive) type III NO synthase is an essential inducer of marrow cell mobilisation in vivo,[6] while the nitric oxide synthase inhibitor, asymmetric dimethylarginine, inhibits the mobilisation, differentiation and function of PCs.[5] Moreover, endogenous NO mediates erythropoietin activity[3] and promotes the hypoxia inducible factor-1-DNA binding that leads to erythropoietin expression.[7]

We investigated, in a consecutive series of patients with documented IHD and no known cause of anaemia, whether impaired NO bioavailability (NOx) might be associated with reduced [Hb], possibly helping to explain the adverse outcomes related to lower Hb levels.

METHODS

Between January and June 2004, 83 patients referred to our Cardiac Care Unit and satisfying our inclusion/exclusion criteria were consecutively enrolled. All patients with documented IHD were eligible for inclusion. Exclusion criteria were age >80 years, left ventricular ejection fraction <30%, lung or liver failure, and known cause of anaemia (recent overt bleeding, congenital or acquired haematological disease, gastrointestinal disorder, moderate-to-severe renal failure, iron or vitamin B deficiency, malignancy). Coronary angiography and echocardiography were performed in all cases. The protocol received ethics committee approval. Informed consent was obtained from all participants.

To achieve an α of 0.05, a β of 80% and an intergroup difference in NOx >10 μM, we estimated a population of 16 patients per group was necessary. The first [Hb] measurement and first white cell count (WCC) were used for analyses. Patients were grouped into those with [Hb] <13 g/dl and those with [Hb] ⩾13 g/dl. The cut-off of 13 g/dl was chosen a priori as representing the median of the lower normal limit of [Hb] in our laboratory for males (14 g/dl) and females (12 g/dl). As per protocol, NOx (μM) and C-reactive protein (CRP, mg/l) were measured in blood taken at least 48 h after the administration of nitrates or the occurrence of haemodynamic instability (see table 1). Plasma aliquots were frozen at −80°C. NOx was assessed as nitrites and nitrates using Griess' reaction (Nitric Oxide Colorimetric Assay, Roche, Mannheim, Germany); this method is less expensive and less time-consuming than mass spectrometry while maintaining a satisfactory correlation with the latter.[8] CRP was assessed by high-sensitivity nephelometry (NCRP-hs, Roche), admission blood glucose, triglycerides, and total and HDL-cholesterol by routine spectrophotometric assays, and WCC and red cell count by automatic electronic analyser. Clinical/biochemical variables were compared by χ^2, Fisher exact, unpaired t or Mann Whitney U tests, as appropriate. Forward stepwise regression was performed with [Hb] < or ⩾13 g/dl as a dichotomous, dependent variable. Independent variables were CRP, age, NOx and any other variable found to associate with [Hb] at a $p \leqslant 0.2$ (WCC, clinical diagnosis, left ventricular hypertrophy and treatment with aspirin). A two-tailed $p<0.05$ was considered statistically significant. Analyses were performed using SPSS version 11.

RESULTS

Patients with [Hb] <13 g/dl did not differ significantly from patients with Hb values ⩾13 g/dl with respect to clinical and biochemical variables (table 1, fig 1), except for NOx and [Hb]. On univariate analysis, NOx was significantly lower in patients with [Hb] <13 g/dl ($p = 0.009$, fig 1). On multivariate regression, NOx remained an independent predictor of [Hb] ($p = 0.01$). Conversely, CRP ($p = 0.33$), WCC ($p = 0.35$), age ($p = 0.89$), clinical diagnosis ($p = 0.94$ for all myocardial infarctions, $p = 0.97$ for ST-elevation infarction, $p = 0.5$ for non-ST-elevation infarction and $p = 0.82$ for unstable angina), left ventricular hypertrophy ($p = 0.28$) and treatment with aspirin ($p = 0.70$) did not significantly predict [Hb]. Both male and female patients with [Hb] <13 g/dl showed significantly lower NOx compared to those with [Hb] ⩾13 g/dl ($p = 0.04$ for both genders).

DISCUSSION

Because lower Hb concentrations are associated with increased cardiovascular risk,[1] a better understanding of the biochemical correlates of blood Hb may have important clinical implications. In this group of patients, reduced NOx independently predicted the presence of [Hb] <13 g/dl. Thus, IHD patients without known causes of anaemia but with lower [Hb] may be at higher risk of adverse cardiovascular events by virtue of lower NOx, with an attending attenuation of NO's vasodilatory, antiplatelet, anti-inflammatory and insulin-sensitising effects. Impaired NOx may also signal reduced erythropoietin activity and reduced circulating PCs, since NO is a major signalling metabolite for erythropoietin and appears to be essential for

Abbreviations: CRP, C-reactive protein; [Hb], haemoglobin concentration; IHD, ischaemic heart disease; NO, nitric oxide; NOx, nitric oxide bioavailability; PC, progenitor cell; WCC, white cell count

Table 1 Baseline characteristics of the study population

	All patients (n = 83)	Patients with [Hb] <13 g/dl (n = 17)	Patients with [Hb] ≥13 g/dl (n = 66)	p
Age, years	62±8	63±10	61±8	0.60
Males, n (%)	69 (83.1)	13 (76.5)	56 (84.8)	0.40
Cardiovascular risk factors, n (%)				
Diabetes	19 (22.9)	5 (29.4)	14 (21.2)	0.50
Hypertension	19 (22.9)	5 (29.4)	14 (21.2)	0.89
Smoking	62 (74.7)	11 (64.7)	51 (77.3)	0.35
Blood glucose, mg/dl	123.1±64.6	147.8±89.8	116.8±54.0	0.20
HDL-cholesterol, mg/dl	41.3±11.9	38.8±11.3	42.1±12.1	0.31
Total cholesterol, mg/dl	188.1±46.9	178.8±56.6	190.6±44.0	0.43
Triglycerides, mg/dl	141.4±63.1	146.6±45.9	140.0±67.4	0.64
Serum creatinine, mg/dl	1.2±1.4	1.0±0.3	1.3±1.5	0.25
WCC ($\times 10^3$/mm^3)	8.8±3.4	9.8±3.4	8.6±3.34	0.18
RCC (10^{12}/l)	4.8±0.8	4.7±0.8	4.8±0.8	0.50
Hb, g/dl*	14.4 (10.2–18.7)	12.3 (10.2–12.9)	14.6 (13–18.7)	0.0001
NOx, μM†	22.4±1.1	15.1±11.0	24.3±16.8	0.009
CRP, mg/l	13.1±30.0	18.9±42.8	11.1±2.5	0.51
Time from admission to NOx and CRP sample, h	97±59	103±47	95±62	0.33
Diagnosis on admission, n (%)				
AMI	32 (38.6)	9 (52.9)	23 (34.8)	0.27
UA	25 (30.1)	2 (11.8)	23 (34.8)	0.08
CSA	26 (31.3)	6 (35.3)	20 (30.3)	0.91
Prior MI, n (%)	35 (42.2)	7 (41.2)	28 (42.4)	0.85
LVEF (%)	50.2±12.3	51.0±9.8	49.9±13.1	0.70
LVH, n (%)	31 (37.3)	3 (17.6)	28 (42.4)	0.09
Coronary artery disease, n (%)				
0 Vessel	11 (13.2)	1 (5.9)	10 (15.2)	0.45
1 Vessel	21 (25.3)	5 (29.4)	16 (24.2)	0.75
2 Vessel	20 (24.1)	4 (23.5)	16 (24.2)	1
3 Vessel	31 (37.3)	7 (41.2)	24 (36.4)	0.9
Medication, n (%)				
ASA	67 (80.7)	16 (94.1)	51 (77.3)	0.17
β-Blockers	56 (67.5)	12 (70.6)	44 (66.7)	0.89
ACE inhibitors	52 (62.7)	13 (76.5)	39 (59.1)	0.29
Diuretics	26 (31.3)	7 (41.2)	19 (28.8)	0.49
Statins	52 (62.7)	11 (64.7)	41 (62.1)	0.93
Ca^{2+} antagonists	19 (22.9)	4 (23.5)	15 (22.7)	1

Continuous values are means±SD, unless otherwise stated. AMI, acute myocardial infarction; ASA, aspirin; CRP, C-reactive protein; CSA, chronic stable angina; Hb, haemoglobin; HDL, high density lipoprotein; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; NOx, nitric oxide bioavailability; RCC, red cell count; UA, unstable angina; WCC, white cell count.
*Median and range; †see fig 1 for individual values, medians, interquartiles and ranges.

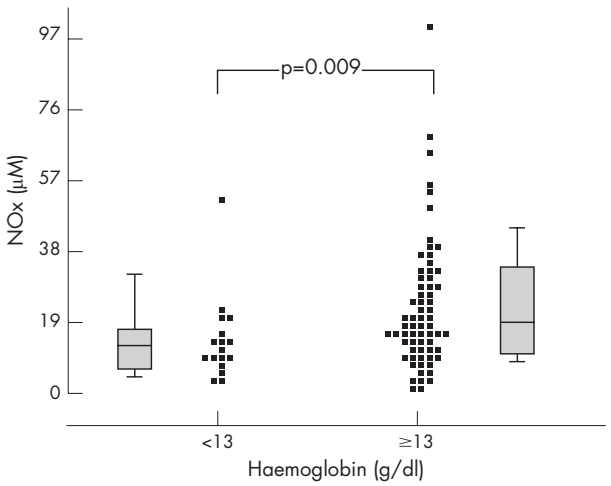

Figure 1 Scattergram of plasma nitrite and nitrate values (NOx) according to haemoglobin concentrations ([Hb]). Patients with [Hb] <13 g/dl had significantly lower median (range) NOx compared to patients with [Hb] ≥13 g/dl: 14.5 (6.1–52.4) μM *v* 19.4 (1.1–103.4) μM (p = 0.009 on univariate analysis and p = 0.01 on multivariate regression). Boxes indicate medians and interquartiles; whiskers are 5th–95th percentiles.

PC function.[3 5 7] Interestingly, a direct relationship between the number of circulating CD34+ cells and Hb concentrations has been reported in patients with IHD,[9] while erythropoietin and circulating PCs are both emerging as potential cardioprotective factors.[4]

Reduced [Hb] in patients with IHD may be ascribed to chronic, low-grade inflammation,[2] related, at least in part, to the high prevalence of vascular risk factors. Indeed, low-grade inflammation is a well-recognised predictor of adverse outcome. In the present investigation, however, [Hb] was not significantly associated with the major risk factors nor with the systemic indices of inflammation, CRP and WCC. Moreover, the relation between [Hb] and NOx found in the present study does not support the mechanism of haematopoietic suppression through enhanced cytokine-induced NO.[2] These observations suggest that reduced [Hb][1] and inflammation may be harbingers of adverse outcomes through separate pathways.

Although our data do not indicate whether the association between NOx and [Hb] is causal or coincidental, the recent discovery of endothelial NO as an essential determinant of PC mobilisation and differentiation[5 6] and our finding of NOx as an independent predictor of [Hb] are consistent with the possibility that reduced NO may contribute to a general depression of haematopoietic function, expressed by reduced circulating PCs[5] and by reduced erythropoiesis.

Some study limitations include the lack of circulating PC or erythropoietin measurements, the relatively small number of patients with [Hb] <13 g/dl and the inability to establish whether reduced NO bioavailability reflects lesser NO synthesis (and if so, from which NO synthase isoform and from which cellular compartment), greater scavenging by reactive oxygen species or impaired interactions with haemoglobin.[10] Nonetheless, to our knowledge, this is the first description of a significant link between reduced [Hb] and impaired NOx in patients with documented IHD. Low [Hb] may predict adverse outcomes in these patients not only through reduced oxygen delivery but also through reduced NOx and, possibly, through scanty PC mobilisation.

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